ABSTRACT...Background: In smooth muscle cells, an increase in cytosolic free calcium concentration is an essential step for the cells to contract. The increase in calcium concentration occurs by influx from the extra cellular medium through calcium ion channels. Calcium channels have potential role in regulation of motility in gastrointestinal tract so there is growing interest in calcium channel blockers as a potential pharmacological approach to the treatment of various gastrointestinal motor disorders. In this study we evaluate the inhibitory effects of verapamil on spontaneous contractions of isolated guinea pig ileum and compare them with inhibitory effects of loperamide. Materials and Methods: Isolated intestinal segments of sixteen guinea pigs were used in this study. Serial dilutions (10-18 - 10-3 gm/ml) of loperamide and verapamil were administered; effects observed and recorded by 7B Grass Polygraph machine. Results: We observed that at lower concentrations, loperamide showed more inhibitory effects than verapamil while at higher concentrations (10-4 and 10-3 gm/ml) verapamil showed more inhibitory effects than loperamide on the contractions of isolated guinea pig ileum. Conclusion: This study gave us a clue that verapamil found a potent inhibitor of small intestinal contractions as loperamide. However one can presume further that calcium channel blocker verapamil acting on calcium channel receptor on GIT will be developed with more specific effects on smooth muscle of intestinal tract.

Key words: Verapamil, Loperamide, Small Intestinal Movements.

INTRODUCTION
Smooth muscles in digestive tract, uterus and uterine tubes under goes slow spontaneous rhythm contractions for peristalsis that can be modulated by autonomic nerves and hormones. Smooth muscle cells contains large amount of regulatory proteins called Calmodulin. Calmodulin is a calcium dependent regulatory protein that plays important roles for contractile activity in smooth muscles and mediates regulation of numerous calcium dependent intracellular events. In smooth muscle in the presence of calcium, a complex is formed between calcium calmodulin and myosin light-chain kinase (MLCK), which then phosphorylates myosin light chain called regulatory chain and initiates cross-bridge cycling and contraction.

To activate the contractile apparatus, Ca\(^{2+}\) must increase globally throughout the cytoplasm.

The Ca\(^{2+}\) utilized for activation of the contractile apparatus enters the cytoplasmic compartment during periods of...
membrane depolarization, mechanical distortion, or stimulation by agonists. Release of Ca\(^{2+}\) from intracellular stores is a second means of increasing [Ca\(^{2+}\)]. After an excitatory event, relaxation and Ca\(^{2+}\) homeostasis are achieved by reuptake of Ca\(^{2+}\) into stores and extrusion into the extra cellular space\(^{17}\). To cause relaxation of smooth muscle contraction, it is necessary to remove the calcium ions from the intracellular fluids surrounding the actin myosin filaments. This removal is achieved by a calcium pump that pumps the calcium ions out of the smooth muscle fibers back into the extra cellular fluid or pumps calcium ions into the sarcoplasmic reticulum\(^{1}\).

There is evidence that there can be significant variation in the degree of participation of extra cellular and intracellular Ca\(^{2+}\) in smooth muscle contraction. Other results indicate that extra cellular Ca\(^{2+}\) participate in spontaneous activity and enters cytosol by L-type voltage-dependent Ca\(^{2+}\) channels\(^{16,20}\). Diarrhoeal diseases caused several million of deaths in the world annually. In developing countries they are the most common causes of morbidity and mortality\(^{14,6}\).

All electrically excitable cells through out the animal kingdom possess Ca\(^{2+}\)-channels\(^{18}\). The blockade of L-type Ca\(^{2+}\) channels by verapamil significantly inhibits the frequency of giant migrating contractions (GMC) during small intestinal inflammation\(^{13}\). Loperamide is piperidine derivative. Its mechanism of action is as of opioid. The chemical structure of loperamide resembles organic calcium channel antagonist drugs such as verapamil. Although these drugs are used predominantly for cardiovascular disorders, they do affect other tissues and organs. Constipation is a major side effect of treatment with calcium antagonists such as verapamil. Loperamide displays pharmacological calcium antagonism in smooth muscle. Their calcium antagonist actions may be responsible in part for antidiarrhoeal effects. The intestinal concentration of loperamide after pharmacologically relevant doses is sufficient to block calcium channels. It is believed that the control of electrolyte and fluid secretion is involved both in etiology of diarrhea and its control by opiates. Calcium regulates the balance between absorption and secretion across the intestinal mucosa. Low intracellular calcium levels favor absorption; rise in intracellular calcium promotes secretion\(^{16}\). Loperamide dose dependently delayed colonic transit and prolonged the evacuation time\(^{12}\). Loperamide also caused the inhibition of spontaneous contractions\(^{18}\).

**PURPOSE OF STUDY**

To study and compare the inhibitory effects of Verapamil (a calcium canal blocker), with Loperamide (a established antidiarrheal opioid) on spontaneous contractions of isolated guinea pig ileum.

**STUDY DESIGN**

Materials and Methods: All experimental works were carried out in the Department of Pharmacology and Therapeutics, Basic Medical Science Institute (B M S I), Jinnah Post Graduate Medical Center (JPMC) Karachi. Drugs (Verapamil and Loperamide) were supplied from ZAFA Pharmaceutical Pakistan limited Karachi. Serial dilutions of all drugs were made from 10\(^{-3}\) to 10\(^{-16}\) gm/ml. Repeated measure design is used to compare the within-series (Concentrations) effect and between series (Drug) effect.

**Nutritional Solution**

The specified Tyrode physiological nutrient solution (contains Sodium Chloride, Potassium Chloride, Calcium Chloride Anhydrous, Magnesium Chloride, Sodium Bicarbonate, Sodium Dihygro Phosphate, Dextrose, and Distilled Water) was used for the perfusion of isolated intestinal segments.

Preparation and Isolation of Small Intestine: Sixteen healthy adult guinea pigs of both sexes (non pregnant), obtained from animal house of JPMC Karachi, weighing 450 – 750 gm. The animals were killed by a blow on the head with hammer. The abdomen was opened by midline incision, where ileum was located. A piece of terminal ileum was separated and transferred to Petri dish containing aerated (oxygenated) Tyrode solution, where it was cleaned from fecal material fat and extra connective tissues. A segment of about 15 – 20 mm long
was taken from isolated ileum, and mounted vertically in inner organ bath (which contains 20 ml Tyrode solution) with the help of tissue holder. It was connected to Polygraph with the help of force displacement transducer, and was subjected to the tension of 1 gm. The nutrition solution was continuously aerated. Temperature of organ bath was maintained at 37°C. Preparations were allowed to equilibrate in Tyrode solution for at least 30 – 45 minutes. The drugs were added in small quantities (1 ml) to inner organ bath according to experimental protocol.

Methodology: Eight experiments were done in each group. In group-I and II, the tissues were subjected to serial dilutions (from 10-18 to 10-3 gm/ml) of Loperamide and Verapamil respectively. Responses were recorded for two minutes for each dilution on Polygraph displacement transducer.

Repeated measure design is used to compare the within-series (Concentrations) effect and between series (Drug) effect.

OBSERVATIONS AND RESULTS
Results were based on the rate and amplitude of ileal contractions.

Rate: When overall we compared Loperamide with Verapamil on the basis of rate of contractions, there was non-significant (p = 0.685) difference observed. But at higher concentrations (10-4gm/ml and 10-3gm/ml) highly significant difference was found with p-value p<0.001. This difference was due to significant interaction effect between drug and concentration (p<0.001).

Amplitude: When Loperamide and Verapamil were compared on the basis of amplitude of contractions in isolated ileum, there was non significant difference observed (p = 0.17) with smaller mean values of Verapamil than Loperamide. But when we compared Loperamide and Verapamil at higher concentration (10-4gm/ml and 10-3gm/ml), a highly significant difference was seen with p-values p<0.001. That difference was due to significant interaction effect between drug and concentration (p<0.001). Fig-1 and 2.

DISCUSSION
Our objective for this study was not to review all that is known about the pharmacology of these drugs (Loperamide and Verapamil) but to investigate a hypothesis for the inhibitory role played by calcium channel blocker verapamil in comparison with loperamide, on spontaneous contractions of ileum. The first finding of our study is that verapamil decreasing both the amplitude and frequency of the contraction as reported recently by Gilani. A.H®. Our observations were in complete agreement with the work of Koe E, Timlioglu O. Who studies the inhibitory effects of verapamil,
diltiazem and nifidipine, and found that all the calcium channel blockers significantly decreased the frequency and amplitude of the spontaneous contractions on isolated duodenum of rat1. Ahmed Samir Kaseem in his study found that loperamide improved the consistency of stool probably through its anti motility effects2. Which also matches our results that loperamide decreases the rate as well as amplitude of contractions in isolated guinea pig ileum.

Daly J. W. and Harper J. said that loperamide is widely used anti diarrheal that primarily acts at nano molar concentration through activation of opioid receptors in the gastro intestinal tract. At some what higher concentrations loperamide blocks (calmodulin activity) calcium channels. Loperamide at micro molar concentration had now been shown to have a remarkable stimulatory effect on the capacitative calcium influx⁴. Our observations are in complete agreement with the results of above study, that at lower concentrations (10⁻⁸ gm/ml) loperamide decreases the rate and amplitude non-significantly, whereas at some what higher concentration (10⁻⁶ gm/ml) loperamide had significant (p<0.01) decreased the rate as well as amplitude (may be due to calcium channel blocking action), while at mili molar (10⁻³ gm/ml) concentration loperamide showed not increase in rate and amplitude of contraction but only its inhibitory response decreased (it may be due to increase influx of calcium). Honda H. et al (1994) worked on isolated stomach and ileum of guinea pig and found that loperamide caused greater inhibition of contraction in the ileum than fundus. He also found that in calcium free solution, loperamide and verapamil had no influence, but markedly inhibited calcium induced contractions⁶. We had also used these two drugs i.e., loperamide and verapamil, both decreased the non significantly rate and amplitude at low concentrations of normal, spontaneous contractions (which are also calcium dependent) in isolated guinea pig ileum. Borrelli Francesca in his study seen that the inhibitory effect of Boswellia serrata gum resin extract (BSE) on acetylcholine – induced contractions was reduced by the L-type Ca²⁺- channel blockers verapamil and nifedipine, but not by the sarcoplasmic reticulum Ca²⁺- ATPase inhibitor cyclopiazonic acid, by the phosphodiesterase type IV inhibitor rolipram or by the lipoxygenase inhibitor zileuton².

CONCLUSION
From above discussion it has been concluded that Loperamide an opioid derivative as well as Verapamil a calcium channel blocker were found potent inhibitors of spontaneous contractions of isolated guinea pig ileum.

After statistically analysis and percentage wise comparison of Loperamide and Verapamil, we observed that at lower concentrations Loperamide showed more inhibitory effects than Verapamil while at higher concentrations (10⁻⁴ gm/ml and 10⁻³ gm/ml) Verapamil showed more inhibitory effects than Loperamide on contractions of isolated guinea pig ileum. “However work remains to evaluate the inhibitory effects of Verapamil in comparison with loperamide on Acetylcholine induced contractions of isolated guinea pig ileum and than established by confirmation on vivo study, so human studies are awaited”.

Copyright© 26 Jan, 2010.

REFERENCES


Ca2+ channel blockade by verapamil inhibits GMCs and diarrhea during small intestinal inflammation. Am. J. Physiol, 1997; 273: 785-794.


Spasmolytic effect of petroselinum crispum (Parsley) on rat's ileum at different calcium chloride concentrations. Pakistan Journal of Biological Sciences 2007;10(22):4036-4042.


The secret of happiness is freedom. The secret of freedom is courage.

Jennifer